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Possible antioxidant and neuroprotective mechanisms of FK506 in attenuating haloperidol-induced orofacial dyskinesia

Amanpreet Singh, Pattipati S. Naidu, Shrinivas K. Kulkarni*

Pharmacology Division, University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh-160014, India

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Abstract

Tardive dyskinesia is a serious motor side effect of chronic neuroleptic therapy. The pathophysiology of this disabling and commonly irreversible movement disorder is still obscure. It may be caused by a loss of dopaminergic cells, due to free radicals as a product of high synaptic dopamine levels. Chronic treatment with neuroleptics leads to the development of abnormal oral movements in rats called vacuous chewing movements. Vacuous chewing movements in rats are widely accepted as an animal model of tardive dyskinesia. Chronic haloperidol (1 mg/kg for 21 days) treatment significantly induced vacuous chewing movements and tongue protrusions in rats, and FK506 (Tacrolimus) {[3*S*-[3*R**[*E*(1*S**,3*S**,4*S**)],4*S**,5*R**,8*S**,9*E*,12*R**,14*R**,15*S**,16*R**,18*S**,19*S**,26*aR**]]-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26*a*-hexadecahydro-5, 19-dihydroxy-3-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylethenyl]-14, 16-dimethoxy-4,10,12, 18-tetramethyl-8-(2-propenyl)-15, 19-epoxy-3*H*-pyrido[2,1-*c*][1,4] oxaazacyclotricosine-1,7,20, 21(4*H*,23*H*)-tetrone, monohydrate} dose dependently (0.5 and 1 mg/kg) reduced these haloperidol-induced movements. Biochemical analysis revealed that chronic haloperidol treatment significantly induced lipid peroxidation and decreased the levels of glutathione and of the antioxidant defense enzymes, superoxide dismutase and catalase, in the brains of rats.

Co-administration of FK506 dose dependently (0.5 and 1 mg/kg) and significantly reduced the lipid peroxidation and restored the decreased glutathione levels induced by chronic haloperidol treatment. It also significantly reversed the haloperidol-induced decrease in brain superoxide dismutase and catalase levels. The major findings of the present study suggest that oxidative stress-induced neuronal death might play a significant role in neuroleptic-induced orofacial dyskinesia. In conclusion, FK506 could be a useful drug for the treatment of neuroleptic-induced orofacial dyskinesia.

Keywords: Oxidative stress; Neurodegeneration; Tardive dyskinesia; Vacuous chewing movement; Neuroleptic; FK506; Neuroprotection

1. Introduction

Neuroleptics are among the most widely prescribed psychotropic drugs. They are usually indispensable in the management of patients with psychotic symptoms and are also useful for severe behavioral disturbances associated with dementia. However, the conventional or typical neuroleptics, such as haloperidol, are associated with a wide range of neurological side effects, the extrapyramidal side effects being the most problematic (Parkinsonism and tardive dyskinesia) (Raja, 1995; Sachdev et al., 1999), of which tardive dyskinesia is among the most serious in terms of frequency, persistence, treatment resistance, overall impact on the well-being of the patients and their caregivers, and medicolegal implications (Jeste et al., 1999). Despite the awareness that neuroleptics could cause extrapyramidal side effects, these drugs remain the most effective means of treating schizophrenia and for the managing behavioral disorders in developmentally disabled individuals. In spite of the high frequency of occurrence and known etiology of chronic neuroleptic treatment, the pathophysiology of tardive dyskinesia is still an enigma. Since the first report of tardive dyskinesia as an adverse effect of chronic neuroleptic therapy (Schonecker, 1957), numerous attempts have been made to unravel the pathological mechanism (s) underlying the development of tardive dyskinesia, but none have yielded promising results. Likewise, various treatments have been tried with limited success (Kulkarni and Naidu, 2001).

* Corresponding author. Tel.: +91-172-534113, +91-172-541142, +91-172-779426; fax: +91-172-779426, +91-172-541142.
E-mail address: skpu@yahoo.com (S.K. Kulkarni).

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Chronic treatment with neuroleptics can cause some neuropathological changes in the central nervous system (Cadet and Kahler, 1994). However, most studies have concentrated on the biochemical or behavioral alterations associated with chronic neuroleptic treatment. The neurodegeneration theory of tardive dyskinesia has gained importance in recent years. According to the neurodegeneration hypothesis, tardive dyskinesia is the consequence of neuroleptic-induced neuronal loss, particularly in the striatum (Cadet and Lohr, 1989). Two interacting mechanisms have been proposed for the pathogenesis of cell damage: excitotoxicity and the production of free radicals.

The involvement of excitotoxicity in acute neuronal damage is well described, but the exact mechanism for the proposed excitotoxicity in chronic neurodegeneration and tardive dyskinesia is still unclear. Modest but sustained elevation of extracellular glutamate levels could be involved (Dawson et al., 1995). An intriguing possibility is that impaired cellular energy metabolism, which is a consequence of classical neuroleptic use, could be involved. It is highly possible that different mechanisms may be responsible for tardive dyskinesia, and that excitotoxicity and oxidative stress may very well act together, since these are closely related processes (Coyle and Puttfarcken, 1993). Furthermore, a disruption of mitochondrial energy production may lead to an increased production of reactive oxygen species (Wallace, 1999). These processes are all induced by the striatal degeneration proposed in the γ-amino butyric acid (GABA) hypothesis (Fibiger and Lloiyd, 1984).

Neuroimmunophilin ligands represent a promising new class of agents that have the potential to address a broad area of therapeutic need in the realm of neurological disorders. These compounds are orally bioavailable, well tolerated and active in a variety of neuropharmacological disease models with once per day oral administration. Steiner et al. (1992) and Hamilton et al. (1997) showed that GPI1046 [-(3-pyridyl)-1-propyl(2*S*)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate] also enhances the regeneration of striatal dopaminergic innervation in 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP)-intoxicated and 6-hydroxydopamine-lesioned mice. Similar findings have been reported for V-10,367, a nonimmunosuppressive neuroimmunophilin ligand (Costantini et al., 1997) and FK506 [3*S*-[3*R** [*E*(1*S**,3*S**,4*S**)],4*S**,5*R**,8*S**,9*E*,12*R**,14*R**,15*S**,16*R**,18*S**,19*S**,26*aR**]]-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26*a*-hexadecahydro-5, 19-dihydroxy-3-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylethenyl]-14, 16-dimethoxy-4,10,12, 18-tetramethyl-8-(2-propenyl)-15, 19-epoxy-3*H*-pyrido[2,1-*c*][1,4] oxaazacyclotricosine-1,7,20, 21(4*H*,23*H*)-tetrone, monohydrate (Gold et al., 1998).

FK506 (tacrolimus), isolated from *Streptomyces tsukubaensis*, is a new FDA-approved immunosupressant with relatively diminished side effects, notably nephrotoxicity (Gold et al., 1995). In addition to its ability to suppress T-cell activity, FK506 has a number of non-immune effects including stimulation of hair growth (Yamamato et al., 1994), liver cell regeneration (Carroll et al., 1994) and wound healing (Francavilla et al., 1989). FK506 suppressed the methamphetamine-induced behavioral responses in mice (Tsukamoto et al., 2001) and showed differential effects on the locomotor activity induced by dopamine D1 and D2/D3 receptor agonists (Sakanoue et al., 2002) via calcineurin inhibition. Recently, Tanaka et al. (2002) reported that chronic treatment with FK506 and its analogue GPI1046 reversed 6-hydroxydopamine-induced dopaminergic dysfunction in mice.

Recent studies (Gold et al., 1994; Lyons et al., 1994) indicated that FK506 also influences the outgrowth of neuronal processes. FK506 increases nerve growth factor (NGF)-induced neurite outgrowth in PC 12 cells and primary cultures of dorsal root ganglion sensory neurons (Lyons et al., 1994). Systemic administration of FK506 enhances the rate of axonal regeneration. To our knowledge, this is the first agent in which systemic exposure has been demonstrated to increase the regeneration rate. FK506 was reported to be a powerful neuroprotective agent in focal ischemia in animals (Sharkey and Butcher, 1994) and shows a wide spectrum of therapeutic potential in various stroke models including permanent, transient, and thrombotic middle cerebral artery occlusion models in rats (Sharkey et al., 2000).

In the present study, we investigated the effect FK506 on neuroleptic-induced orofacial dyskinesia in rats, a potential animal model for tardive dyskinesia.

2. Materials and methods

2.1. Animals

Male Wistar rats, bred in the Central Animal House facility of Panjab University and weighing between 180 and 220 g, were used. The animals were housed under standard laboratory conditions, maintained on a 12-h light and dark cycle (lights on at 0700 h), and had free access to food and water. Animals were acclimatized to laboratory conditions before the test. Each animal was used only once in the experiments. All experiments were carried out between 0900 and 1500 h. The experimental protocols were approved by the Institutional Animal Ethics Committee and conducted according to the Indian National Science Academy Guidelines for the use and care of experimental animals.

2.2. Induction of orofacial dyskinesia

Haloperidol (1 mg/kg i.p.) was given chronically to rats for a period of 21 days to induce oral dyskinesia (Naidu and Kulkarni, 2001a,b, 2002; Naidu et al., 2002). All the behavioral assessments were carried out 24 h after the last dose of haloperidol.

2.3. Behavioral assessment of orofacial dyskinesia

On the test day, rats were placed individually in a small (30 × 20 × 30 cm) Plexiglas observation chamber for the assessment of oral dyskinesia. Animals were allowed 10 min to get used to the observation chamber before behavioral assessment. The number of vacuous chewing movements and tongue protrusions was scored by an observer with the help of hand-operated counter. In the present study, vacuous chewing movements are referred as single mouth openings in the vertical plane not directed toward physical material. If tongue protrusion or vacuous chewing movements occurred during a period of grooming, they were not taken into account. Counting was stopped whenever the rat began grooming, and restarted when grooming stopped. Mirrors were placed under the floor and behind the back wall of the chamber to permit observation of oral dyskinesia when the animal faced away from the observer. The behavioral parameters of oral dyskinesia were measured continuously for 5 min. In all experiments, the scorer was unaware of the treatment given to the animals (Naidu and Kulkarni 2001a,b, 2002; Naidu et al., 2002).

2.4. Biochemical estimations

On the 22nd day of haloperidol treatment, the animals were killed by decapitation immediately after behavioral assessments. The brains were removed (without cerebellum), and rinsed with cold isotonic saline and weighed. A 10% (w/v) tissue homogenate was prepared in 0.1 M phosphate buffer (pH 7.4). The post-nuclear fraction for catalase assay was obtained by centrifugation of the homogenate at 1000 × *g* for 20 min, at 4 °C; for other enzyme assays, it was centrifuged at 12,000 × *g* for 60 min at 4 °C.

2.4.1. Lipid peroxidation assay

The quantitative measurement of lipid peroxidation in brains was performed according to the method of Wills (1966). The amount of malondialdehyde formed was measured by the reaction with thiobarbituric acid at 532 nm using a Perkin-Elmer lambda 20 spectrophotometer. The results are expressed as nmol of malondialdehyde/mg protein using the molar extinction coefficient of the chromophore (1.56×10^5 M^{-1} cm^{-1}).

2.4.2. Estimation of reduced glutathione

Reduced glutathione in the brain was estimated according to the method of Ellman (1959). A sample (0.75 ml) of homogenate was precipitated with 0.75 ml of 4% sulfosalicylic acid. The samples were centrifuged at 1200 × *g* for 15 min at 4 °C. The assay mixture contained 0.5 ml of supernatant and 4.5 ml of 0.01 M (in 0.1 M phosphate buffer, pH 8.0) DTNB (5-5′-Dithio Bis-(2-Nitobenzoic acid)). The yellow color developed was read immediately at 412-nm spectrophotometrically. The results are expressed as nmol of GSH per mg protein.

2.4.3. Superoxide dismutase activity

Superoxide dismutase activity was assayed according to the method of Kono (1978), by which the reduction of nitrazoblue tetrazolium by hydroxylamine hydrochloride is inhibited by superoxide dismutase and is measured at 560-nm spectrophotometrically. Briefly, the reaction was initiated by the addition of hydroxylamine hydrochloride to the reaction mixture containing hydroxylamine hydrochloride and post-nuclear fraction of brain homogenate. The results are expressed as units/mg protein, where one unit of enzyme is

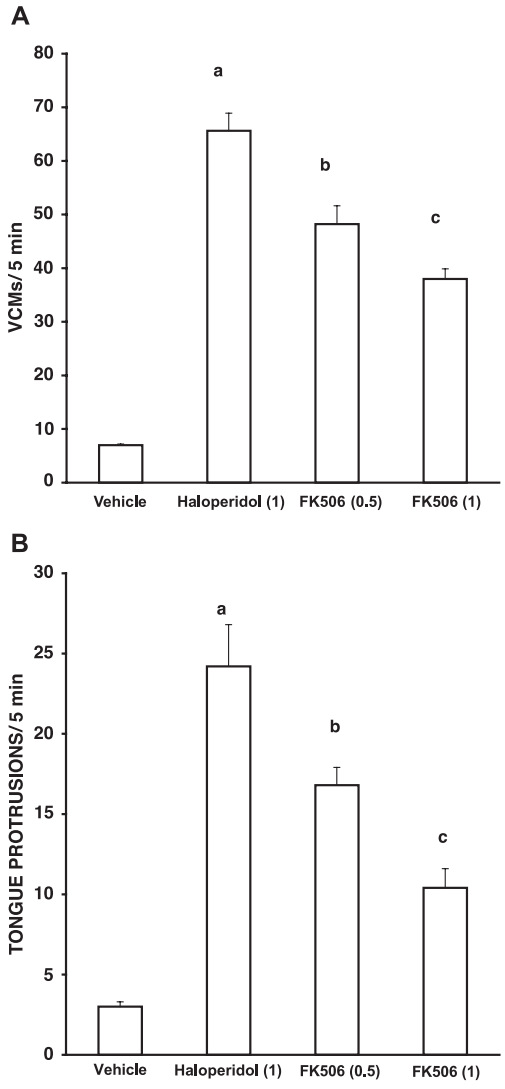

Fig. 1. Effect of co-administration of FK506 on vacuous chewing movements (A) and tongue protrusions (B) induced by chronic (21 days) of haloperidol treatment. Values expressed as means ± S.E.M. $^{a}P < 0.05$ as compared to the vehicle-treated control group. $^{b}P < 0.05$ as compared to haloperidol- or FK506 (1 mg)-treated groups. $^{c}P < 0.05$ as compared to haloperidol- or FK506 (0.5 mg)-treated groups (ANOVA followed by Tukey's test).

defined as the amount of enzyme inhibiting the rate of reaction by 50%.

2.4.4. Catalase activity

Catalase activity was assayed by the method of Luck (1971), by which the breakdown of H_2O_2 is measured at 240 nm. Briefly, the assay mixture consisted of 3 ml of H_2O_2–phosphate buffer (1.25×10^{-2} H_2O_2 m) and 0.05 ml of supernatant of brain homogenate (10%), and the change in absorbance was recorded at 240-nm spectrophotometrically. Enzyme activity was calculated using the millimolar extinction coefficient of H_2O_2 (0.07). The results are expressed as μmol H_2O_2 decomposed/min/mg protein.

2.4.5. Protein estimation

The protein content was measured according to the method of Lowry et al. (1951), using bovine serum albumin as standard.

2.5. Drugs and treatment schedule

The following drugs were used in the present study. Haloperidol (Serenace® inj., Searle India, India) was diluted with distilled water. FK506 (Archer Chemicals, Mumbai, India) was suspended in 0.25% carboxymethylcellulose (CMC). Haloperidol was administered intraperitoneally in a constant volume of 0.5 ml/100 g of body weight of rat. FK506 was given by the per oral route in a constant volume of 0.5 ml/100 g of body weight of rat. Animals were divided into six groups. First group received vehicle, the second group received haloperidol plus vehicle for FK506, the third group received haloperidol plus FK506 0.5 mg/kg, the fourth group received haloperidol plus FK506 1.0 mg/kg, and the fifth and sixth groups received FK506 0.5 and 1 mg/kg alone, respectively. FK506 was administered 10 min before haloperidol administration for a period of 21 days and behavior was assessed 24 h after the last dose. Drug doses were selected on the basis of previous studies conducted in our laboratory and those reported in the literature.

Table 1
Effect of co-administration of FK506 on the lipid peroxidation in the rat brain induced by chronic (21 days) of haloperidol administration

Treatment (mg/kg)	Lipid peroxidation (nmol MDA/mg protein)
Vehicle	1.183 ± 0.071
Haloperidol (1)	3.613 ± 0.123[a]
FK506 (0.5)	2.412 ± 0.106[b]
FK506 (1)	1.982 ± 0.125[b]

Values expressed as means ± S.E.M. for six animals (ANOVA followed by Tukey's test).
[a] $P<0.05$ as compared to vehicle-treated group.
[b] $P<0.05$ as compared to haloperidol-treated group.

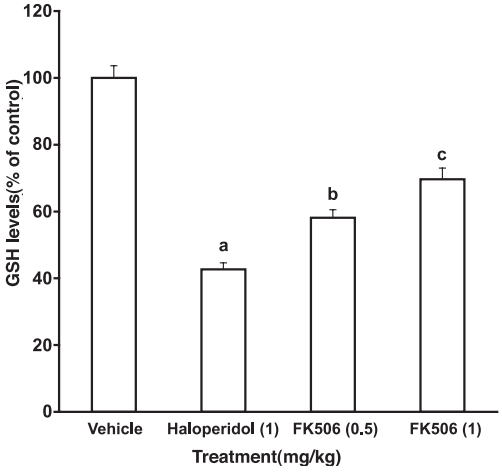

Fig. 2. Effect of co-administration of FK506 on glutathione (GSH) depletion induced by chronic (21 days) of haloperidol treatment. Values expressed as % response of the vehicle-treated control group. Each value represents the mean value for six animals. [a]$P<0.05$ as compared to the vehicle-treated control group. [b]$P<0.05$ as compared to haloperidol- or FK506 (1 mg)-treated groups. [c]$P<0.05$ as compared to haloperidol- or FK506 (0.5 mg)-treated groups (ANOVA followed by Tukey's test).

2.6. Statistical analysis

One specific group of rats was assigned to one specific drug treatment condition and each group comprised six rats ($n=6$). All the values are expressed as means ± S.E.M. The data were analyzed by using analysis of variance (ANOVA) followed by Tukey's test. In all tests, the criterion for statistical significance was $P<0.05$.

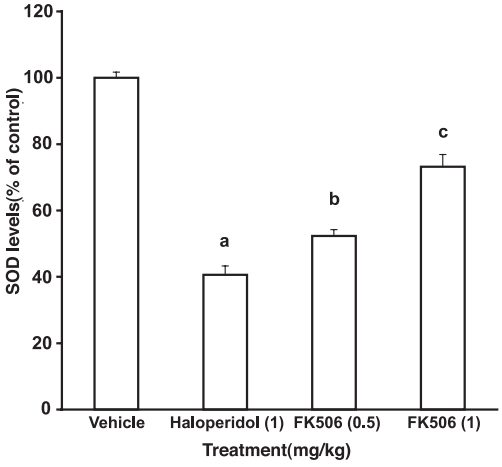

Fig. 3. Effect of co-administration of FK506 on the depletion of brain levels of the antioxidant enzyme superoxide dismutase (SOD) induced by chronic (21 days) of haloperidol treatment. Values expressed as the % response of the vehicle-treated control group. Each value represents the mean value for six animals. [a]$P<0.05$ as compared to the vehicle-treated control group. [b]$P<0.05$ as compared to haloperidol- or FK506 (1 mg)-treated groups. [c]$P<0.05$ as compared to haloperidol- or FK506 (0.5 mg)-treated groups (ANOVA followed by Tukey's test).

3. Results

3.1. Effect of FK506 on haloperidol-induced vacuous chewing movements and tongue protrusions

Chronic haloperidol (1 mg/kg) treatment significantly produced vacuous chewing movements and tongue protrusions in rats. The vehicle-treated control animals did not exhibit such behaviors. Chronic co-administration of FK506 along with haloperidol dose dependently (0.5 and 1 mg/kg) suppressed the haloperidol-induced vacuous chewing movements (Fig. 1A) and tongue protrusions (Fig. 1B). FK506 per se did not cause any significant behavioral changes (data not shown).

3.2. Effect of FK506 on the brain malondialdehyde level in chronic haloperidol-treated rats

Chronic haloperidol for 21 days induced lipid peroxidation, as indicated by a significant increase in brain malondialdehyde levels as compared those to of vehicle-treated rats. Co-administration of FK506 (0.5 and 1 mg/kg) along with haloperidol significantly reversed the extent of lipid peroxidation as compared to that in haloperidol only treated rats (Table 1).

3.3. Effect of FK506 on the brain glutathione levels in chronic haloperidol-treated rats

Statistical analysis of brain glutathione levels showed a significant difference between vehicle-treated and haloperidol-treated rats. Co-administration of FK506 (0.5 and 1 mg/kg) significantly reversed the haloperidol-induced (Fig. 2) decrease in brain glutathione levels.

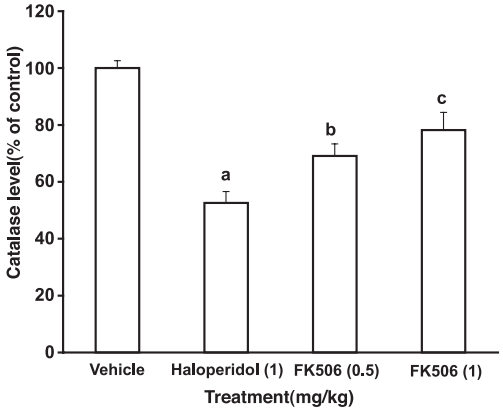

Fig. 4. Effect of co-administration of FK506 on the depletion of brain levels of the antioxidant enzyme catalase induced by chronic (21 days) of haloperidol treatment. Values expressed as the % response of the vehicle-treated control group. Each value represents the mean value for six animals. [a]$P<0.05$ as compared to the vehicle-treated control group. [b]$P<0.05$ as compared to haloperidol- or FK506 (1 mg)-treated groups. [c]$P<0.05$ as compared to haloperidol- or FK506 (0.5 mg)-treated groups (ANOVA followed by Tukey's test).

3.4. Effect of FK506 on the brain antioxidant enzyme levels in chronic haloperidol-treated rats

Chronic haloperidol-treated rats showed decreased levels of the antioxidant enzymes superoxide dismutase and catalase in brain homogenates. Co-administration of FK506 (0.5 and 1 mg/kg) significantly reversed the haloperidol-induced decrease in brain superoxide dismutase (Fig. 3) and catalase (Fig. 4) levels as compared to those as haloperidol-treated rats.

4. Discussion

In the present study, chronic haloperidol-treated animals showed increased frequencies of vacuous chewing movements and tongue protrusions as compared to vehicle-treated control animals. Chronic treatment with FK506 significantly attenuated the induction of haloperidol-induced vacuous chewing movements and tongue protrusion in a dose-dependent fashion. Rats treated with FK506 alone did not show any significant behavioral changes as compared with vehicle-treated control rats (data not shown).

Existing evidence indicates that an excessive production of free radicals is associated with chronic neuroleptic use and might contribute to the onset of tardive dyskinesia and other movement disorders, such as dystonias and Parkinsonism (Cadet et al., 1986). Neuroleptics act by blocking dopamine receptors (Creese et al., 1976). This blockade increases catecholamine turnover, which leads to excess production of free radicals, especially in catecholamine-rich areas such as the basal ganglia. Because of the high oxidative metabolism in these regions, neurons are particularly vulnerable to membrane lipid peroxidation and cell death. Free radicals are highly reactive with specific cellular components and have cytotoxic properties (Coyle and Puttfarcken, 1993; Ravindranath and Reed, 1990), and neuronal loss in the striatum has been reported in animals treated chronically with neuroleptics (Nielsen and Lyon, 1978).

In the present study, chronic haloperidol-treated animals showed decreased levels of glutathione and increased levels of lipid peroxidation products as compared to vehicle-treated control animals. Chronic haloperidol-treated animals had low levels of detoxifying enzymes, such as superoxide dismutase and catalase, as compared to vehicle-treated control animals suggesting possible induction of free radical generation by chronic haloperidol treatment. FK506 dose dependently decreased the elevated level of lipid peroxidation products in haloperidol-treated animals, elevated the cellular defense mechanisms, such as glutathione, and also induced the production of superoxide dismutase and catalase, further suggesting the role of free radicals in the pathophysiology of haloperidol-induced orofacial dyskinesia and the possible antioxidant action of FK506. Rats treated with FK506 alone did not show any significant

biochemical changes as compared with the vehicle-treated control group (data not shown).

FK506 is reported to be a powerful neuroprotective agent in focal ischemia in animals (Sharkey and Butcher, 1994), showing a wide spectrum of therapeutic potential in various stroke models including permanent, transient, and thrombotic middle cerebral artery occlusion in rats (Sharkey et al., 2000). FK506 has neuroprotective potential in animals. It reduces infarct size following cerebral ischemia (Sharkey and Butcher, 1994) with improvement in motor skills (Sharkey et al., 1996), and increases nerve regeneration following crush of peripheral nerve fibers and prevents central nervous system neurons from dying following axotomy. Neutrite outgrowth potentiation underlies this stimulating effect. FK506 is reported to protect dopaminergic and GABAergic neurons from the effect of cerebral ischemia. FK506 also reduces microglial activation in the substantia nigra (Brecht et al., 2000). So far, the exact mechanism for the neuroprotection afforded by this compound is not well known, although various putative mechanisms have been postulated (Sharkey et al., 2000).

FK506 activity is mediated by a family of proteins termed FK506-binding proteins (FKBP). The 12-kDa receptor, FKBP-12 (Harding et al., 1989; Siekierka et al., 1990), is a ubiquitous protein that has been highly conserved throughout evolution (Siekierka et al., 1990) and FKBP appears to be most likely involved in FK506 activity (Sigal and Dumont, 1992). FK506 binding protein is present in neuronal tissue (Steiner et al., 1992) and mRNA levels of FK506 binding protein are reported to be increased following axotomy (Lyons et al., 1995). FK506 has been shown to inhibit the protein phosphatase calcineurin. This protein phosphatase is found not only in T cells, where it may play a role in immunosupression, but also in neuronal tissue. Interestingly, the substrate of calcineurin is the growth-associated protein, GAP-43. GAP-43 is well established as playing an important role in nerve regeneration. FK506 increases the phosphorylation of GAP-43 and activated GAP-43, resulting in increased nerve regeneration (Steiner et al., 1992; Steiner and Hamilton, 1997; Gold et al., 1995).

Calcineurin inhibition appears to be a common feature in FK506-induced neuroprotection (Sharkey et al., 2000). Calcineurin has been implicated in a wide variety of physiological and pathological processes (Morioka et al., 1999), and there are a number of ways by which FK506 could affect neuroprotection by inhibiting calcineurin. Calcineurin has been shown to modulate the activity of both nitric oxide synthase (NOS) and NMDA receptors (Lieberman and Mody, 1994), thus providing a mechanism by which calcineurin inhibitors exert neuroprotection. FK506 has been shown to affect NMDA-stimulated neurotransmitter release and to inhibit glutamate-induced NOS activity in vitro (Dawson et al., 1993; Toung et al., 1996; Steiner et al., 1996).

Calcineurin inhibition may still be a valid target for FK506-mediated neuroprotection in the light of more recent data implicating the involvement of calcineurin in the apoptotic cascade by its interaction with Bcl-2 family proteins. FK506 prevents apoptosis by inhibiting BAD (Bcl-2 family) dephosphorylation and translocation (Wang et al., 1999) or via Bcl-2 sensitive pathways, findings which support this hypothesis (Shibaki and McKeon, 1995; Wolvetang et al., 1996).

In peripheral organs, FK506 has been shown to affect various components of the inflammatory cascade, by inhibiting neutrophil infiltration (Kawano et al., 1991), reducing free radical production by monocytes and polymorphonuclear (Lockhart et al., 1998), and altering cytokine expression (Losa Garcia et al., 1998). FK506 reduced lipid peroxidation and increased striatal glutathione levels (Tanaka et al., 2002). This has led to speculation that the neuroprotective actions of FK506 may, at least in part, be mediated by an attenuation of the free radical production and inflammatory response (Jean et al., 1998).

The dose used in the present study (1 mg/kg) appears to be insufficient to prevent the rejection of neuronal transplants in the rat (Sakai et al., 1991). However, FK506 administration produced no visible adverse effects to preclude continued daily administration of the drug.

The results of the present study confirm that FK506 has an antioxidant activity. This activity appears particularly relevant not only for the understanding of the molecular mechanisms that underlie the action of FK506, but also represents a valid rationale for the use of FK506 in the prevention of neuroleptic-induced orofacial dyskinesia.

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References

Brecht, S., Schwarze, K., Mielke, K., Winter, C., Christner, C., Fischer, G., Herdegen, T., 2000. Neuroprotective immunophilin-ligand mechanisms following cerebral ischemia and nerve fiber injury. In: Gold, B.G., Fischer, G., Herdgen, T. (Eds.), Immunophilins in the Brain. Prous Science, Barcelona, Spain, pp. 129–146.

Cadet, J.L., Kahler, L.A., 1994. Free radial mechanism in schizophrenia and tardive dyskinesias. Neurosci. Biobehav. Rev. 18, 457–767.

Cadet, J.L., Lohr, J.B., 1989. Possible involvement of free radicals in neuroleptic-induced movement disorders: evidence from treatment of tardive dyskinesia with vitamin E. Ann. N.Y. Acad. Sci. 4, 37–46.

Cadet, J.L., Lohr, J.B., Jeste, D.V., 1986. Free radicals and tardive dyskinesias. Trends Neurosci. 9, 107–108.

Carroll, P.B., Rilo, H.L.R., Elmagd, K.A., Johnson, N., Carter, C., Wright, H., Jegasothy, B., Starzl, T.E., Van Thiel, D.H., 1994. Effects of tacrolimus (FK506) in dystrophic epidermolysis bullosa: rationale and preliminary results. Arch. Dermatol. 130, 1457–1458.

Costantini, L.C., Chaturvedi, P., McCaffrey, P., Deacon, T.W., 1997. Neuroprotective and regenerative effects of immunophilin ligands in animal model of Parkinson's disease. Abstr.-Soc. Neurosci. 23, 1452.

Coyle, J.T., Puttfarcken, P., 1993. Oxidative stress glutamate and neurodegenerative disorders. Science 262, 689–695.

Creese, G., Burt, D., Synder, S.I.L., 1976. Dopamine receptor binding and pharmacological potencies and antischizophrenic drugs. Science 192, 481–483.

Dawson, T.M., Steiner, J.P., Dawson, V.L., Dinerman, J.L., Uhl, G.R., Snyder, S.H., 1993. Immunosupressant FK506 enhances phosphorylation of nitric oxide synthase and protects against glutamate neurotoxicity. Proc. Natl. Acad. Sci. U. S. A. 90, 9808–9812.

Dawson Jr., R., Beal, M.F., Bondy, S.C., Di Monte, D.A., Isom, G.E., 1995. Excitotoxins, aging, and environmental neurotoxins: implications for understanding human neurodegenerative diseases. Toxicol. Appl. Pharmacol. 134, 1–17.

Ellman, G.L., 1959. Tissue sulfhydryl groups. Arch. Biochem. Biophys. 82, 70–77.

Fibiger, H.C., Lloiyd, K.G., 1984. Neurobiological substrates of tardive dsykinesia: the GABA hypothesis. Trends Neurosci. 7, 462–464.

Francavilla, A., Barone, M., Todo, S., Zeng, Q., Porter, K.A., Starzl, T.E., 1989. Augmentation of rat liver regeneration by FK506 compared with cyclosporine. Lancet 1, 1248–1249.

Gold, B.G., Strom-Dickson, T., Austin, D.R., 1994. The immunosupressant FK506 increases functional recovery and nerve regeneration following peripheral nerve injury. Restor. Neurol. Neurosci. 6, 287–296.

Gold, B.G., Katoh, K., Strom-Dickerson, T., 1995. The immunosupressant FK506 increases the rate of axonal regeneration in rat sciatic nerve. J. Neurosci. 15, 7509–7516.

Gold, B.G., Gordon, H.S., Zeleny-Pooley, M., Wang, M.S., 1998. FK506 produces regeneration, but not neuroprotection, of nigrostriatal dopaminergic neurons in MPTP-treated mice. Brain Pathol. 8, 583.

Hamilton, G.S., Huang, W., Connolly, M.A., et al., 1997. FKB12-binding domain analogues of FK506 are potent, nonimmunosupressive neurotropic agents in vitro and promote recovery in mouse model of Parkinson's disease. Bioorg. Med. Chem. Lett. 7, 1785–1790.

Harding, M.W., Galat, A., Uehling, D.E., Schreiber, S.L., 1989. A receptor for the immunosupressant FK506 is a *cis-trans*-peptidyl-propyl isomerase. Nature 341, 758–760.

Jean, W.C., Spellman, S.R., Nussbaum, E.S., Low, W.C., 1998. Reperfusion injury after focal cerebral ischemia: the role of inflammation and therapeutic horizon. Neurosurgery 43, 1382–1396.

Jeste, D.V., Lacro, J.P., Baily, A., Rockwell, E., Harris, J.M., Calliguri, M.P., 1999. Lower incidence of tardive dyskinesia with rispiridone compared with haloperidol in older patients. J. Am. Geriatr. Soc. 47, 716–719.

Kawano, K., Kim, Y.I., Goto, S., Ono, M., Kobayashi, M., 1991. A protective effect of FK506 in ischemically injured rat livers. Transplantation 52, 143–145.

Kono, Y., 1978. Generation of superoxide radical during autooxidation of hydroxylamine and an assay for superoxide dismutase. Arch. Biochem. Biophys. 186, 189–195.

Kulkarni, S.K., Naidu, P.S., 2001. Tardive dyskinesia: an update. Drugs of Today 37, 97–119.

Lieberman, D.N., Mody, I., 1994. Regulation of NMDA channel function by endogenous Ca^{2+}-dependent phosphatase. Nature 369, 235–239.

Lockhart, B.P., Cressy, K.C., Lepagnol, J.M., 1998. Suppression of nitric oxide formation by tyrosine kinase inhibitors in murine N9 microglia. Br. J. Pharmacol. 123, 879–889.

Losa Garcia, J.E., Mateos Rodriuez, F., Jimenez Lopez, A., et al., 1998. Effect of cyclosporin A on inflammatory cytokine production by human alveolar macrophages. Respir. Med. 92, 722–728.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin-Phenol reagent. J. Biol. Chem. 193, 265–275.

Luck, H., 1971. Catalase. In: Bergmeyer, H.U. (Ed.), In Methods of Enzymatic Analysis. Academic Press, New York, pp. 885–893.

Lyons, W.E., George, E.B., Dawson, T.M., Steiner, J.P., Snyder, S.H., 1994. Immunosupressant FK506 promotes neurite outgrowth in cultures of PC12 cells and sensory ganglia. Proc. Natl. Acad. Sci. U. S. A. 91, 3191–3195.

Lyons, W.E., Steiner, J.P., Snyder, S.H., Dawson, T.M., 1995. Neuronal regeneration enhances the expression of the immunophilin FKBP-12. J. Neurosci. 15, 2985–2994.

Morioka, M., Hamada, J., Ushio, Y., Miyamoto, E., 1999. Potential role of calcineurin for brain ischemia and traumatic injury. Prog. Neurobiol. 58, 1–30.

Naidu, P.S., Kulkarni, S.K., 2001a. Possible involvement of prostaglandins in haloperidol-induced orofacial dyskinesia in rats. Eur. J. Pharmacol. 430, 295–298.

Naidu, P.S., Kulkarni, S.K., 2001b. Effect of 5-HT1A and 5-HT2A/2C receptor modulation on neuroleptic-induced vacuous chewing movements. Eur. J. Pharmacol. 428, 81–86.

Naidu, P.S., Kulkarni, S.K., 2002. Dopamine–glutamate modulation of haloperidol-induced vacuous chewing movements in rats. Brain Pharm. 1, 147–158.

Naidu, P.S., Singh, A., Kulkarni, S.K., 2002. Possible antioxidant mechanisms in carvedilol reversal of haloperidol-induced orofacial dyskinesia. Br. J. Pharmacol. 136, 193–200.

Nielsen, E.B., Lyon, M., 1978. Evidence for cell loss in corpus striatum after long-term treatment with a neuroleptic drug (flupenithixol) in rats. Psychopharmacology 59, 85–89.

Raja, M., 1995. Tardive dystonia: prevalence, risk factors and comparison with tardive dyskinesia in a population of two hundred acute psychiatric in patients. Eur. Arch. Psychiatry Clin. Neurosci. 245, 145–151.

Ravindranath, V., Reed, D.J., 1990. Glutathione depletion and formation of glutathione protein mixed disulfide following exposure of brain mitochondria to oxidative stress. Biochem. Biophys. Res. Commun. 169, 150–158.

Sachdev, P., Saharov, T., Cathcart, S., 1999. The preventive role of antioxidants (selegiline and vitamin E) in a rat model of tardive dyskinesia. Biol. Psychiatry 46, 1672–1681.

Sakai, K., Date, I., Yoshimoto, Y., Arisawa, T., Nakashima, H., Furuta, T., Asari, S., Ohmoto, T., 1991. The effect of a new immunosuppressive agent, FK506, on xenogeneic neural transplantation in rodents. Brain Res. 565, 167–170.

Sakanoue, M., Mori, N., Takei, N., Kawai, M., Tani, K., Suzuki, K., Iwata, Y., Sekine, Y., Ashby Jr., C.R., Minabe, Y., 2002. Tacrolimus, a specific inhibitor of calcineurin, modifies the locomotor activity of quinpirole, but not that of SKF82958, in male rats. Eur. J. Pharmacol. 438, 93–97.

Schonecker, M., 1957. Ein eigentumliches syndrom in oralen bereich bei megaphen application (Letter). Nervenarzt 28, 35.

Sharkey, J., Butcher, S.P., 1994. Immunophilins mediate the neuroprotective effects of FK506 in focal cerebral ischemia. Nature 371, 336–339.

Sharkey, J., Crawford, J.H., Butcher, S.P., Marston, H.M., 1996. Tacrolimus (FK506) ameliorates skilled motor deficits produced by middle cerebral artery occlusion in rats. Stroke 27, 2282–2286.

Sharkey, J., Jones, P.A., McCarter, J.F., Kelly, J.S., 2000. Calcineurin inhibitors as neuroprotectants. Focus of tacrolimus and cyclosporin. CNS Drugs 13, 1–13.

Shibaki, F., McKeon, F., 1995. Calcineurin functions in Ca^{2+}-activated cell death in mammalian cells. J. Cell Biol. 131, 735–743.

Siekierka, J.J., Wiederrecht, G., Greulich, H., Boulton, D., Hung, S.H.Y., Cryan, J., Hodges, P.J., Sigal, N.H., 1990. The cytosolic-binding protein for the immunosupressant FK506 is both a ubiquitous and highly conserved peptidyl-propyl *cis-trans* isomerase. J. Biol. Chem. 265, 21011–21015.

Sigal, N.H., Dumont, F.J., 1992. Cyclosporin A, FK506, and rapamycin: pharmacologic probes of lymphocyte signal transduction. Annu. Rev. Imnmunol. 10, 519–560.

Steiner, J.P., Dawson, T.M., Fotuhi, C., Glatt, C., Snowman, A.M.M., Cohen, N., Snyder, S.H., 1992. High brain densities of the immunophilin FKBP co-localized with calcineurin. Nature 358, 584–587.

Steiner, J.P., Dawson, T.M., Fotuhi, M., Snyder, S.H., 1996. Immunophilin regulation of neurotransmitter release. Mol. Med. 2, 325–333.

Steiner, J.P., Hamilton, G.S., Ross, D.T., et al., 1997. Neurotrophic immunophilin ligands stimulate structural and functional recovery in neurodegenerative animal models. Proc. Natl. Acad. Sci. U. S. A. 94, 2019–2024.

Tanaka, K., Yoshioka, M., Miyazaki, I., Fujita, N., Ogawa, N., 2002. GPI1041 prevents dopaminergic dysfunction by activating glutathione system in the mouse striatum. Neurosci. Lett. 321, 45–48

Toung, T.J., Bhardwaj, A., Dawson, V.L., Dawson, T.M., Traystman, R.J., Hurn, P.D., 1996. Neuroprotective FK506 does not alter in vivo nitric oxide production during ischemia and early reperfusion in rats. Stroke 30, 1279–1285.

Tsukamoto, T., Iyo, M., Tani, K., Sekine, Y., Hashimoto, K., Ohashi, Y., Suzuki, K., Iwata, Y., Mori, N., 2001. The effects of FK506, a specific calcineurin inhibitor, on methamphetamine-induced behavioral changes and its sanitization in rats. Psychopharmacology 158, 107–113.

Wallace, D.C., 1999. Mitochondrial diseases in man and mouse. Science 283, 1482–1488.

Wang, H.G., Pathan, N., Ethell, I.M., et al., 1999. Ca^{2+}-induced apoptosis through calcineurin dephosphorylation of BAD. Science 284, 339–343.

Wills, E.D., 1966. Mechanism of lipid peroxide formation in animal tissues. Biochem. J. 99, 667–676.

Wolvetang, E.J., Larm, J.A., Moutsoulas, P., Lawen, A., 1996. Apoptosis induced by inhibitors of the plasma membrane NADH-oxidase involves Bcl-2 and calcineurin. Cell Growth Differ. 7, 1315–1325.

Yamamato, S., Jiang, H., Kato, R., 1994. Stimulation of hair growth by topical application of FK506, a potent immunosuppressive agent. J. Invest. Dermatol. 102, 160–164.